Blue mussel (*Mytilus edulis*) settlement patterns on antifoulant treated salmon nets and its implications for recycling used salmon nets for mussel spat collection in Integrated Multi-trophic Aquaculture (IMTA).

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ABSTRACT

Lander, T.R., Shaw, R.K., Robinson, S.M.C. and Martin, J.D. 2009. Blue mussel (*Mytilus edulis*) settlement patterns on antifoulant treated salmon nets and its implications for recycling used salmon nets for mussel spat collection in Integrated Multi-trophic Aquaculture (IMTA). Can.Tech. Rep. Fish. Aquat. Sci. 2849: vi + 20 p., 2009.

The recycling of waste in aquaculture systems is the foundation for Integrated Multi-trophic Aquaculture (IMTA). Integrated systems implement lower trophic levels to extract excess organic and inorganic wastes from the fin-fish farms. In the Bay of Fundy we have been using the blue mussel (*Mytilus edulis*) as an organic extractive component in IMTA systems. In order to successfully implement mussels as a full commercial crop in the IMTA system, a consistent collection source for juveniles ('spat' or 'seed') must be identified. The potential for reusing old salmon nets treated with the antifoulant cuprous oxide, for mussel spat collection was examined in this study. The settlement density and shell length of blue mussels was measured on newly treated (NT), previously treated (PT) and untreated (UT) collector panels cut from commercial salmon nets, to assess how mussel spat collection efficiency was affected by commercial cuprous oxide treatment. Collectors units were deployed at 4 locations for approximately 5 months. Significant differences were found between treatments for mean spat density (MSD) in 3 of 4 locations in decreasing order of UT>PT>NT. Reduced settlement on NT and PT panels was attributed to the inhibitory effects of cuprous oxide on mussel settlement. Although PT panels collected fewer spat than UT panels, the numbers were still adequate for commercial spat collection. Mean shell length (MSL) was not significantly different between treatments but a trend of decreased shell length in the order of NT>PT>UT was observed in 3 of the 4 locations. Self-thinning is a possible explanation for the inverse relationship observed between MSL and MSDs. Based on these results, we feel there is a potential for recycling used salmon nets for mussel collection as PT panels continued to collect large numbers of mussel spat while inhibiting unwanted foulers with lingering antifoulant properties. Regional differences in spat settlement density and mussel size were also found.

Keywords: Blue Mussel, Mytilus edulis, settlement, IMTA, spat collection.

RÉSUMÉ

Lander, T.R., Shaw, R.K., Robinson, S.M.C. et J.D. Martin, « Blue mussel (*Mytilus edulis*) settlement patterns on antifoulant treated salmon nets and its implications for recycling used salmon nets for mussel spat collection in Integrated Multi-trophic Aquaculture (IMTA) », Rapp. tech. can. sci. halieut. aquat.,. 2849: vi +20 p., 2009.

L'aquaculture multi-trophique intégrée (AMTI) repose sur le recyclage des déchets dans les systèmes d'aquaculture. Les systèmes intégrés incluent des niveaux trophiques bas pour extraire des déchets organiques et inorganiques excédents des sites d'élevage. Dans la baie de Fundy, nous avons utilisé la moule bleue (*mytilus edulis*) comme composante organique d'extraction dans les systèmes d'AMTI. Afin d'intégrer avec succès les moules en tant que culture commerciale complète dans les systèmes d'AMTI, il faut déterminer une source constante de captage de juvéniles (« naissains » ou « semence »). Dans cette étude, nous avons examiné la possibilité de réutiliser des filets à saumon, traités avec de l'oxyde de cuivre antisalissure, afin de capter les naissains de moule. Nous avons mesuré la densité du frai et la longueur des coquilles des moules bleues sur les surfaces de captage traitées nouvellement (TN), prétraitées (PT) et non traitées (NT), découpées à partir des filets à saumon commerciaux, afin d'évaluer l'efficience des captages de naissains de moules touchés par un traitement à l'oxyde de cuivre antisalissure commercial. Les unités de captage ont été déployées dans 4 emplacements pour une période d'environ 5 mois. Nous avons relevé d'importantes différences entre les types de traitement pour la densité moyenne des naissains (DMN) à 3 des 4 emplacements dans un ordre décroissant, soit NT>PT>TN. La réduction de la fixation sur les surfaces TN et PT s'explique par les effets inhibitoires de l'oxyde de cuivre sur les fixations des moules. Bien que les surfaces PT aient capté moins de naissains que les surfaces NT, leur nombre est suffisant pour le captage commercial. La longueur moyenne des coquilles (LMC) ne différait pas de façon importante entre les traitements, mais nous avons observé une tendance de diminution de la longueur des coquilles dans l'ordre de TN>PT>NT à 3 des 4 emplacements. L'auto-éclaircie expliquerait la relation inverse observée entre les LMC et les DMN. Selon ces résultats, nous pensons qu'il peut être possible de recycler les filets de saumon pour capter les moules, puisque les surfaces PT continuent de capter un grand nombre de naissains de moule tout en inhibant les salissures indésirables grâce à des propriétés antisalissures persistantes. Nous avons également trouvé des différences régionales en ce qui a trait à la densité de la fixation des naissains et à la taille des moules.

Mots clés : Moule bleue, m*ytilus edulis*, fixation, AMTI, captage de naissain.

INTRODUCTION

Integrated Multi-trophic Aquaculture (IMTA) is a concept based on recycling waste from aquaculture sites to promote sustainability in the aquaculture industry. While the primary focus of IMTA is the recycling of nutrients using extractor species, there is the desire to utilize other forms of waste resulting from the farm in operation that may be of use for other applications. Of particular interest is the recycling of used salmon nets which are typically treated with cuprous oxide as an antifoulant, for the collection of mussel spat.

Aquaculture sites use the antifoulant cuprous oxide almost exclusively in the treatment of commercial salmon nets (Braithwaite et al. 2006). The antifoulant acts by releasing a biocide into the water which creates a layer of toxic material around the treated area to prevent the settlement of fouling organisms (Thomas et al. 1999; Douglas-Helders et al. 2003). In the case of the blue mussel, physiological and behavioural responses to the antifoulant deter the organism from settling on the substrate (Wisely 1962; Scott and Major 1972). However, defense mechanisms possessed by the blue mussel have provided an explanation for the continued, but reduced, settlement on treated salmon net cages (Davenport and Manley 1978; Harrison et al. 1983). Over time the effectiveness of the treatment declines and the nets are either retreated or discarded according to the condition of the net. As of yet, there are few uses for discarded nets. This study attempts to assess the collection efficiency, defined as mean spat density (MSD) and mean shell length (MSL) of mussels on salmon nets untreated (UT), newly treated (NT), and previously treated (PT) with cuprous oxide to determine whether or not the recycling of these nets for mussel collection is a viable option for IMTA.

MATERIALS AND METHODS

COLLECTOR DESIGN AND PROTOCOL

A $1m^2$ frame of PVC pipe was constructed to hold 9-$32cm^2$ panels (1cm mesh size) of commercial salmon net (Figure 1). Cable ties were used to attach 3 NT, 3 UT and 3 PT panels to polypropylene lines used to divide the collector unit into equal parts. Two-0.23kg tension weights and a chain were located beneath the collector unit to maintain vertical orientation and minimize tangling, and a swivel was placed atop and beneath the collector unit to enable movement with the current. Tongue Shoal, Crow Island, Bliss Harbour, and Bocabec (Figure 2) were the sites selected for collector deployment due to their representation of 3 different oceanographic areas (Robinson et al.1996, Buzeta 2007). On August 10, 2006 two collector units were deployed at a depth of 3 m at each site, with enough rope to maintain this depth despite changing tides. The experimental duration was approximately 5 months.

COLLECTION AND ENUMERATION OF MUSSELS

Collector units were sampled commencing January 10th, 2007 (Table 1). Each individual panel was bagged and labeled according to its location, treatment and unit number and kept on ice until enumeration of settled individuals in the lab. In order to remove fouling organisms the panels were submerged in a solution of diluted bleach (1:5 bleach:water mixture) for approximately 3 hours. They were then washed through a 10cm PVC pipe with a 1mm mesh bottom to remove unwanted organisms and mussels under the desired size of 1.0mm. Large mussel counts were split using a Folsom plankton splitter (Figure 3) and the appropriate correction factors were applied to the final density values. The mussels were then placed on a grided dissecting tray and enumerated. Mussel shell lengths and widths were measured using Image Pro Plus (Version 5.1).

DATA ANALYSIS

Mean shell length (MSL) and mean spat density (MSD) was calculated for each treatment and location and statistically tested using a one way ANOVA (Version 10.0, SPSS Inc.). MSD and MSL values from Units 1 and 2 in each location were combined to increase the sample size thus improving the statistical power. MSD values could not be combined from Tongue Shoal due to significant heterogeneity of variance in PT panels. Overall trends in MSD and MSL by treatment and location factors were compared using a one way ANOVA. For these analyses all PT, UT and NT panels were combined for panel treatment differences, and all panels per location (all types) were combined for location effects. MSL and shell length:width ratio frequency distributions were determined for each location using all treatment replicates. Length:width ratio was used as a possible indication of the mussel species collected since the shell shape of *Mytilus trossulus* is more elongated and shell shape of the *Mytilus edulis* is eccentric (Innes and Bates, 1999).

RESULTS

TREATMENT

Panels collected significantly fewer mussels in decreasing order of UT>PT>NT in Crow Island, Bliss Harbour, and Bocabec with the exception of PT and NT panels from Bocabec which showed no significant difference (Figure 4). MSD values from units 1 and 2 from Tongue Shoal showed no significant differences between treatments. No significant differences in MSL were found between treatments in each of the 4 locations, although a trend of decreasing shell length in order of NT>PT>UT was observed (Figure 5). However, an overall relationship between mean mussel density and treatment was significant when treatment replicate panels were combined over all four locations (Figure 6a), as total settlement declined in the following order: UT>PT>NT. The inverse relationship was true for mussel size (Figure 6b) with the largest mussels being found

on the NT panels and the smallest were on UT panels, with PT panels experiencing an intermediate MSL.

LOCATION

Significant differences found between locations based on treatment type did not show great consistency (Figure 7). Although Tongue Shoal collected the most spat overall, there was not a strong trend between the locations with intermediate MSDs (Figure 7). Significant differences were not found for MSL between locations (Figure 8).

Significant regional differences in both mussel density and MSL were evident when the means of all panel replicates (combining the 2 collector units) for each of the four experimental locations were calculated. Units collected significantly fewer mussels in decreasing order of Tongue Shoal>Crow Island>Bocabec>Bliss Harbour (Figure 9a). At three of these sites (Tongue Shoal, Crow Island and Bocabec), mean MSL decreased with an increase in overall settlement (Figure 9b). However this relationship was not present at Bliss Harbour location, which experienced the lowest mussels density yet a decreased MSL compared to Bocabec, and equal to Crow Island, whose overall settlement was significantly higher.

The shell length frequency distribution (Figure 10) showed that Tongue Shoal collected the smallest mussel spat at the highest frequency relative to the other sites which followed similar distribution patterns (Figure 10). The length:width ratio frequency distribution showed no differences between sites and was unimodal (Figure 11).

DISCUSSION

In this study, a trend in mussel settlement was found between the 3 panel treatments. PT panels were intermediate collectors relative to the other panel treatments supporting the potential for recycling these nets in the collection of mussel spat which is an important activity required for establishing the mussel culture component in IMTA systems. Regional differences in spat settlement density and mussel size were also found for the Southwest Bay of Fundy.

TREATMENT

As Cu levels in sea water rise above the normal level of 3-10ppm (Chou et al. 1999) a mussel's ability to regulate its accumulation decreases to a point where sub-lethal or lethal effects can be observed (Harrison et al. 1983) resulting in a decrease in settlement. Newly treated panels likely would have had a greater release rate of Cu ions compared to PT and UT panels which would support the observed decrease in mussel settlement with cuprous oxide exposure. Even without direct contact to Cu ions

it has been shown that physiological and behavioural responses can occur to prevent settlement on treated substrate (Wisely 1962; Chou et al. 1999). Wisely (1962) found that behavioural changes, such as turning and retracting into the shell occurred in the mussel, *Mytilus planulatus*, before reaching cuprous oxide antifoulant paint. Mussels were observed to draw a current of water back towards the larvae enabling the detection of the Cu ions prior to contact. It was suggested that the paint prevented mussel settlement due to the retraction of the foot upon contact, as the foot is required for successful attachment to the substrate. Although mussel response to Cu was not assessed in this study, it is likely that a combination of these physiological and behavioural responses to Cu exposure was a major contributor to the reduction in mussel settlement on NT net panels.

While Cu exposure on NT panels resulted in reduced settlement relative to PT and UT panels, approximately 2000 to 160,000 ind./m^2 were collected, depending on the location, indicating a degree of tolerance to the level of copper treatment on the net panels used. Similar results were found in Braithwaite et al. (2006) when the fouling biomass and species composition on control and cuprous oxide treated collectors was investigated. It was shown that blue mussels were among those taxa capable of settling on the substrate with cuprous oxide. Blue mussels have also shown a greater tolerance to Cu exposure relative to other bivalves (Beaumont et al. 1987) which is likely due to the presence of defense mechanisms such as mucous, metallothionein-like-proteins (MLPs), shell closure, and the binding of Cu in vesicles (Scott and Major 1972; Davenport and Manley 1978; Harrison et al. 1983). These defense mechanisms enable the mussel to remove and/or detoxify Cu before concentrations reach an intolerable level. The observed settlement on NT panels may be attributed to these defense mechanisms which make the environment suitable enough for successful, although reduced, settlement.

Previously treated nets were found to be intermediate in effectiveness as mussel collectors. In order for copper antifoulants to maintain their effectiveness Cu ions must be liberated at a rate of 10µg/cm^2/day (Wisely 1962). Approximately twice the number of mussels was found on PT panels compared to NT panels demonstrating that this rate was not maintained during immersion. However, an observed reduction in fouling organisms and a decrease in settlement by approximately 15%, relative to UT panels, may indicate that a slower rate of Cu release was present which facilitated these mild antifoulant properties. The trade off between minimizing unwanted foulers and maximizing mussel collection is important to consider in mussel collection as fouling organisms can be difficult to remove during the stripping and grading processes (Boghen 1995). Based on our results, it appears as though PT panels are capable of collecting mussel spat comparable to UT panels while maintaining mild antifoulant properties capable of minimizing the settlement of unwanted biofoulers such as tunicates, algae, and other bivalve species.

UT panels collected the greatest number of mussels in all locations with the exception of Unit 2 in Tongue Shoal. This was likely due to the absence of cuprous oxide. However, the fouling of other organisms appeared to be the greatest on these

panels as well which made the stripping of mussels from the net extremely difficult and inefficient. Under these conditions mussel sorting was not possible since mussels were bound together by fouling organisms and could not be separated without the use of bleach. Despite collecting the most mussels, UT nets would not be effective for the collection of mussel spat as separation and removal would be greater and hence less cost efficient for the grower.

Mixed results have been found regarding the shell growth of bivalves when exposed to Cu treatments (Paul and Davies 1986; Beaumont et al. 1987). While Paul and Davies (1986) found an increase in scallop growth and no effects on adult oyster and scallop growth, Beaumont et al. (1987) found that blue mussels and *Pecten maximus* were negatively affected by increasing Cu concentrations. We did not find any significant differences between MSL with the exception of Crow Island in which two significantly lower MSL were found on UT panels (Figure 5). In a commercial setting this is important for determining the time at which a sockable size of 5-30mm in length is reached (Boghen 1995). In this study, mussels were collected at approximately 4mm in length, just below the sockable size. A longer immersion period would have been beneficial to determine the full duration required to meet the size requirements. In addition a slight inverse relationship between shell length and mussel density was observed in this study and should be investigated further to assess if this is a consistent trend. This may be pertinent information in determining the trade off between maximizing collection without jeopardizing shell size.

The inverse relationship between shell length and density has been found in mussels and has been considered analogous to the process of self thinning evident in plants (Hughes and Griffiths 1988; Frechette et al. 1992; Petraitis 1995; Alumno-Bruscia et al. 2000; Guinez 2005). Self thinning is a process whereby intraspecific competition, such as reduced food availability per individual (Petraitis 1995), causes mortality. In turn continued growth of survivors produces an inverse relationship between individual mass and density (Hughes and Griffiths, 1988), as was found in this study (Figure 4 and 5). Taking this into consideration, it is likely that the MSD of UT panels will be the first of the 3 panel treatments to plateau or decline as a result of these self thinning effects. It may be beneficial in the future to assess the settlement of these panels on a temporal scale to observe the density changes and determine the point at which populations decline. In addition the effects of self-thinning must strongly be considered to determine the desired balance between density and shell length.

LOCATION

Duration of mussel collection differed from 1-2 weeks between each site which likely influenced the results of the MSD and MSL from each site. This may have contributed to the inconsistent significant differences between sites. However, when all locations were compared in terms of overall mussel numbers, regional heterogeneity of settlement density was seen. These differences may be due to oceanographic

specificity of the different areas which were not measured in this study. However, densities at all locations were high enough to support commercial spat needs, making the use of PT salmon nets as settlement substrate for IMTA, universal in all parts of the bay.

SIZE FREQUENCY DISTRIBUTION

There was little variance between locations with respect to the size of collected mussels. In addition, the length:width frequency distribution must be interpreted with caution as the identification of *M.edulis* and *M. trosilus* and their hybrids is most often carried out with the use of genetic markers (Koehn et al. 1984; Comensana et al. 1999; Toro et al. 2004). Although it has been shown that shell morphology of *M.edulis*, eccentric, and *M. trosilus*, elongated, differs, it is not until the mussels have reached a larger size that these variations in shell shape become apparent (Innes and Bates 1999). Therefore, the mussels collected in this study may not have reached the size at which a length:width ratio would be a true representation of the species differentiation. In order to determine if the collected mussels were a mix of *M.edulis* and *M. trosilus*, a hybrid of the two, or a single species, genetic testing would have been required which was out of the realm of this study.

The results in this study indicate that PT panels are adequate collectors relative to UT panels and may provide mild antifoulant properties to minimize unwanted fouling organisms. This is an attractive feature as stripping and grading of mussels can be impeded by these organisms thus making the collected spat unusable (Boghen 1995). In this study, mussels were collected at approximately 4mm in length which is just smaller than the sockable mussel size of 5-30mm (Boghen 1995). This should be taken into account when assessing the number of spat collected since these densities will decrease as the mussels grow to a sockable size. In future studies it would be ideal to immerse treated panels for a longer period of time to allow mussels to grow to a sockable length to account for these density changes. In addition, a quantitative assessment of the fouling collected on these panels would be an asset to determine if the trade off between the number of mussels collected and fouling organisms is worth considering when selecting a collector type. The recycling of used commercial salmon nets may be a viable option in IMTA systems based on the MSD of mussels collected on PT panels. This innovative use of discarded aquaculture equipment would not only be an economic advantage to those who adopt it, but it would be another step for IMTA towards improving sustainability in the aquaculture industry.

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TABLES

Table 1. Deployment and collection dates for collector units at each location.

Site	Deployment Date	Collection Date
Crow Island	August 10, 2006	January 3, 2007
Tongue Shoal	August 10, 2006	January 15, 2007
Bliss Harbour	August 10, 2006	January 23, 2007
Bocabec	August 18, 2006	January 29, 2007

FIGURES

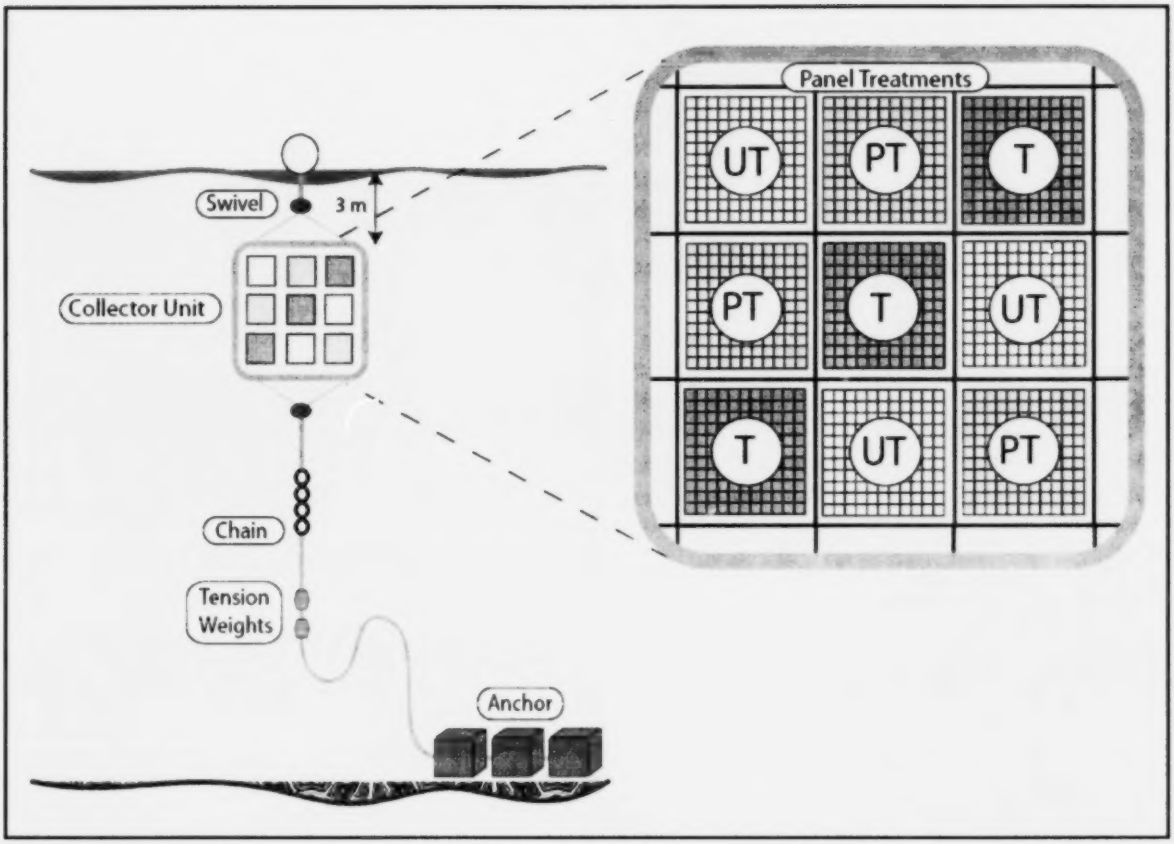

Figure 1: Collector unit set-up.

Figure 2: Map of Passamaquoddy Bay, New Brunswick showing 2 collector units at each location for blue mussel spat collection

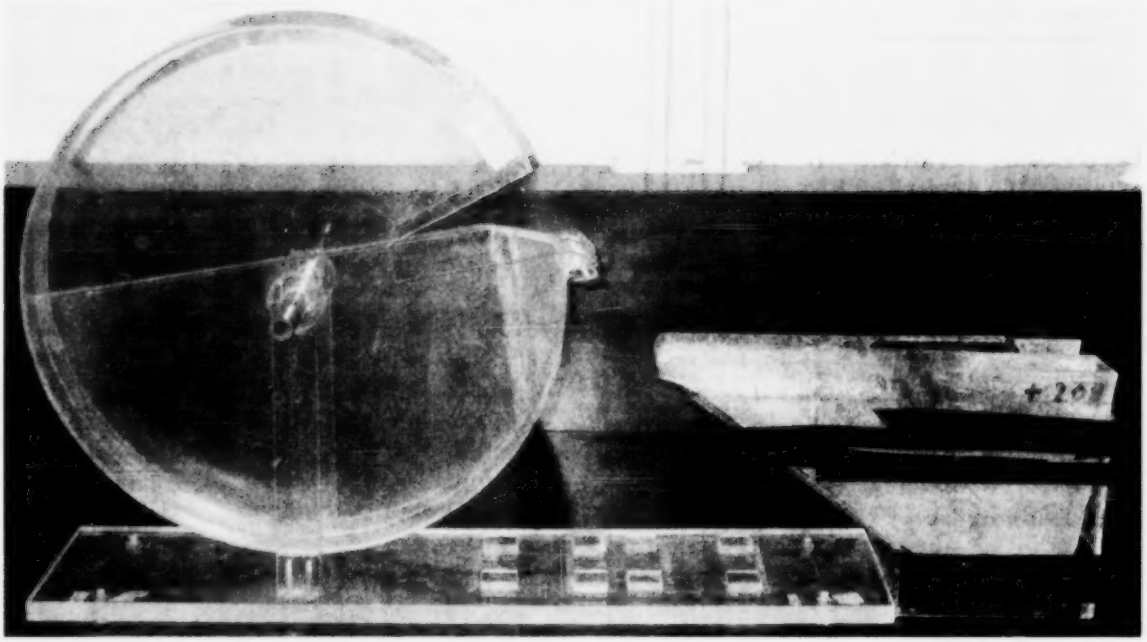

Figure 3: Folsom phytoplankton splitter

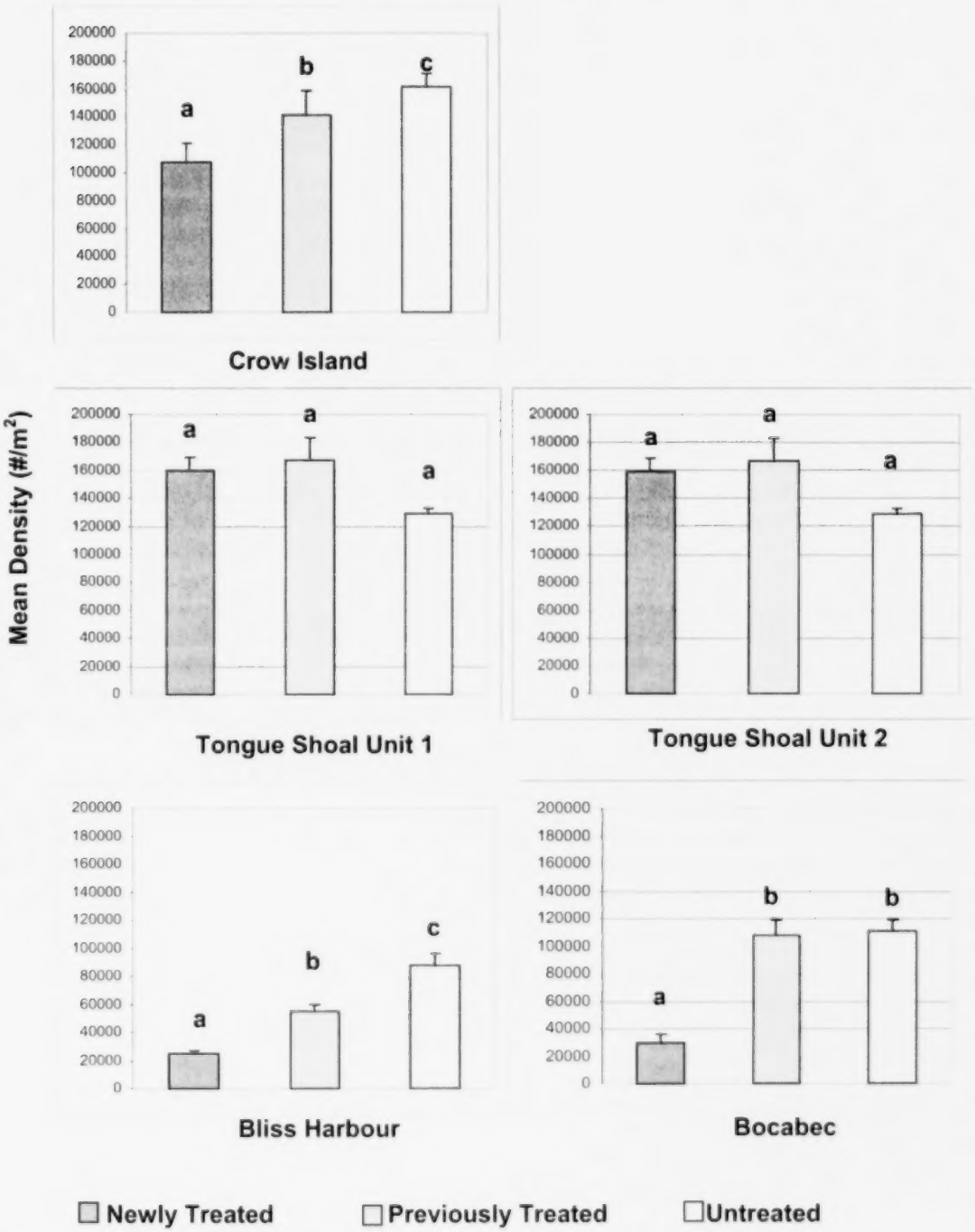

Figure 4: Mean density (#/m^2) of NT, PT, and UT panels was tested for each location using a one way ANOVA. Different letters represents significant differences between treatments; n = 6 for Crow Island, Bocabec, and Bliss Harbour; n = 3 for unit 1 and 2 at Tongue Shoal.

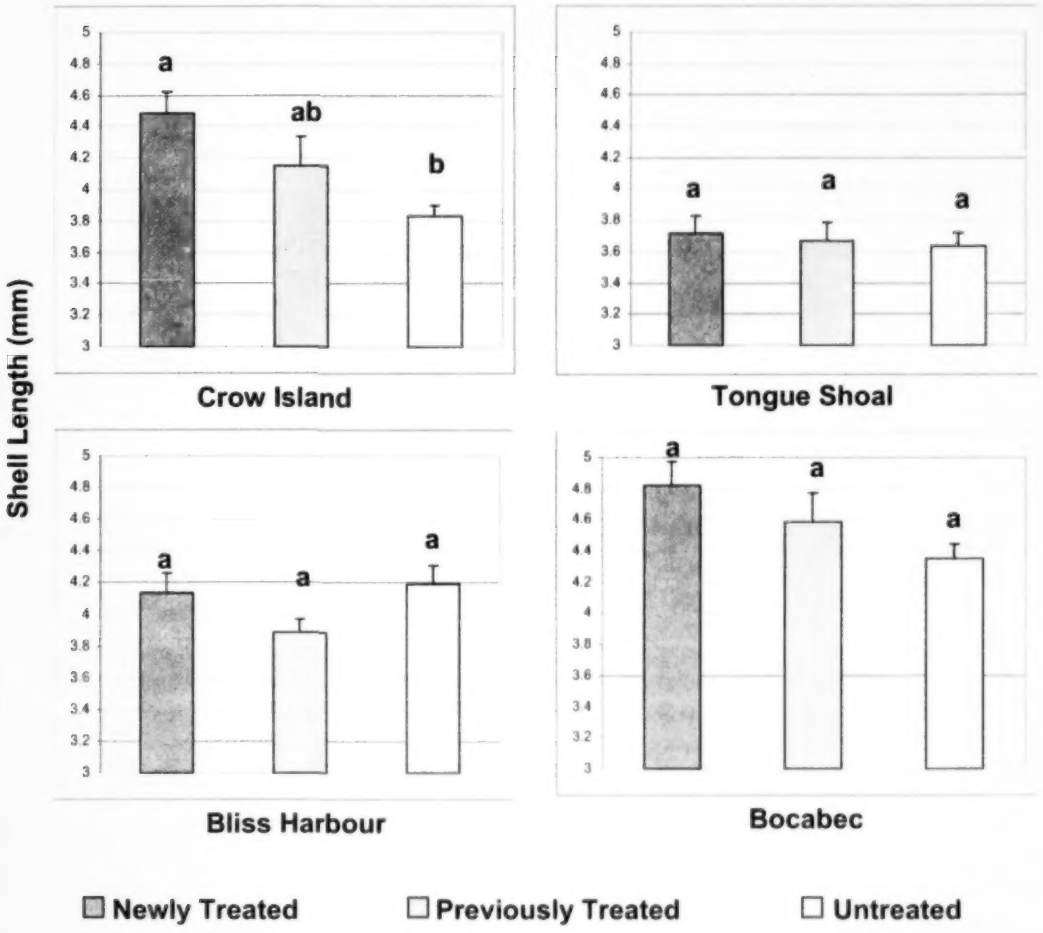

Figure 5: Mean shell lengths (mm) of NT, PT, and UT panels at Crow Island, Tongue Shoal, Bliss Harbour and Bocabec were tested using a one way ANOVA. Different letters represents significant differences between treatments (n = 6 for each treatment).

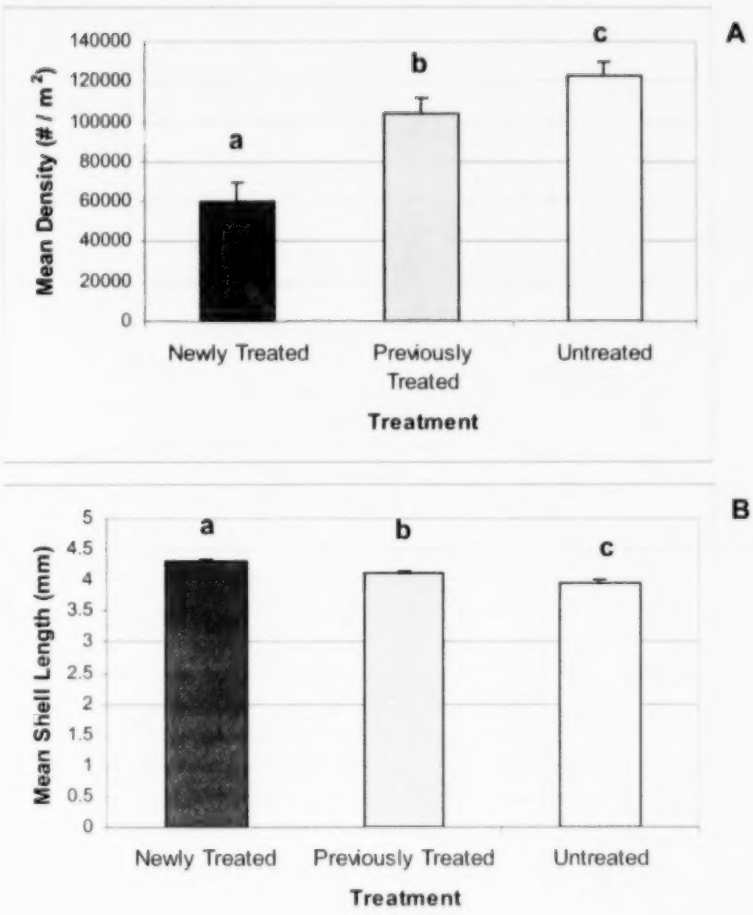

Figure 6: A - mean density of settled mussels (#/m^2) for NT, PT and UT panels combined over all test locations. Different letters represents significant differences between treatments (n = 18) using a one way ANOVA.

B - mean shell length (mm) of NT, PT and UT panels combined for all test locations. Different letters represents significant differences between treatments (n = 18) using a one-way ANOVA.

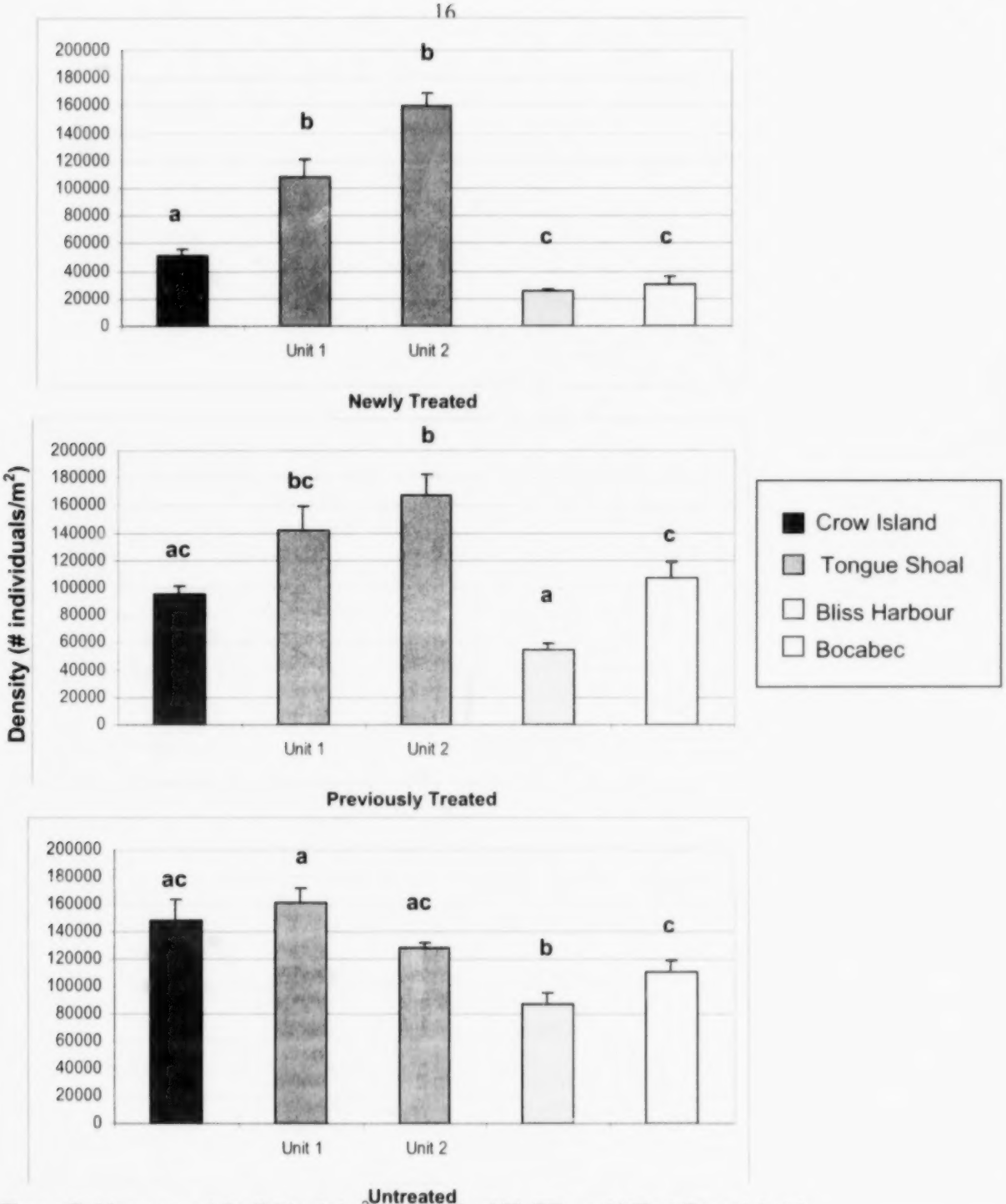

Figure 7: Mean count (individuals/m^2) values for NT, PT, and UT at Crow Island, Units 1 and 2 at Tongue Shoal, Bliss Harbour, and Bocabec. Letters represents significant differences between treatments (n = 6 for Crow Island, Bliss Harbour and Bocabec; n = 3 for units 1 and 2 at Tongue Shoal.

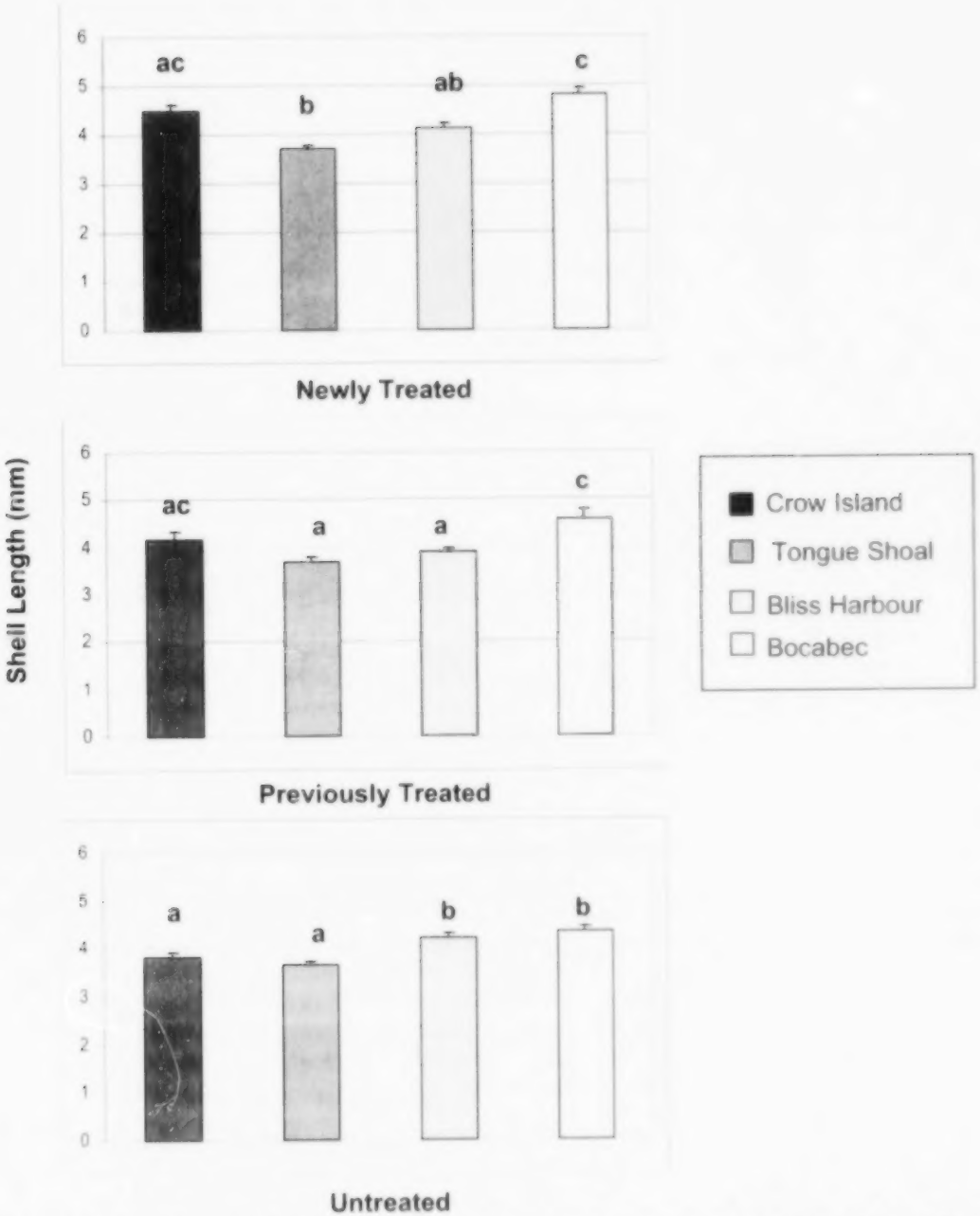

Figure 8: Shell lengths for NT, PT, and UT panels at Crow Island, Tongue Shoal, Bliss Harbour and Bocabec. Different letters represents significant differences between treatments (n = 6 for Crow Island, Bliss Harbour and Bocabec; n = 3 for Unit 1 and 2 at Tongue Shoal).

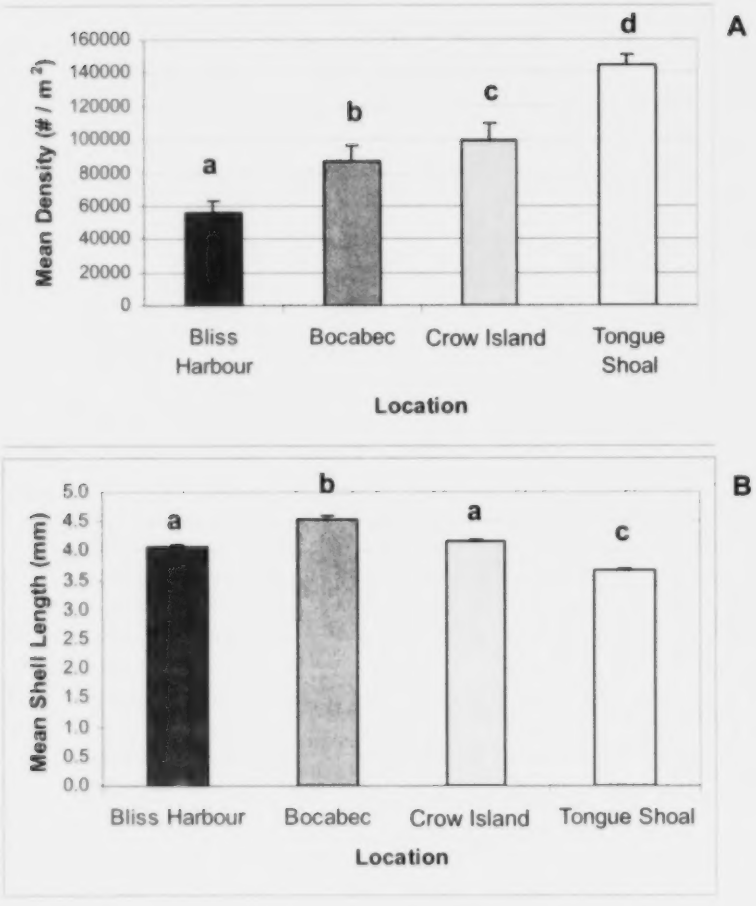

Figure 9: A – mean settlement density of settled mussels (mm) from NT, PT UT panels combined for each test location. Different letters represents significant differences between locations (n = 18) using a one way ANOVA.

B – mean shell length (mm) of NT, PT and UT panels combined for each test location. Different letters represents significant differences between treatments (n = 18) using a one way ANOVA.

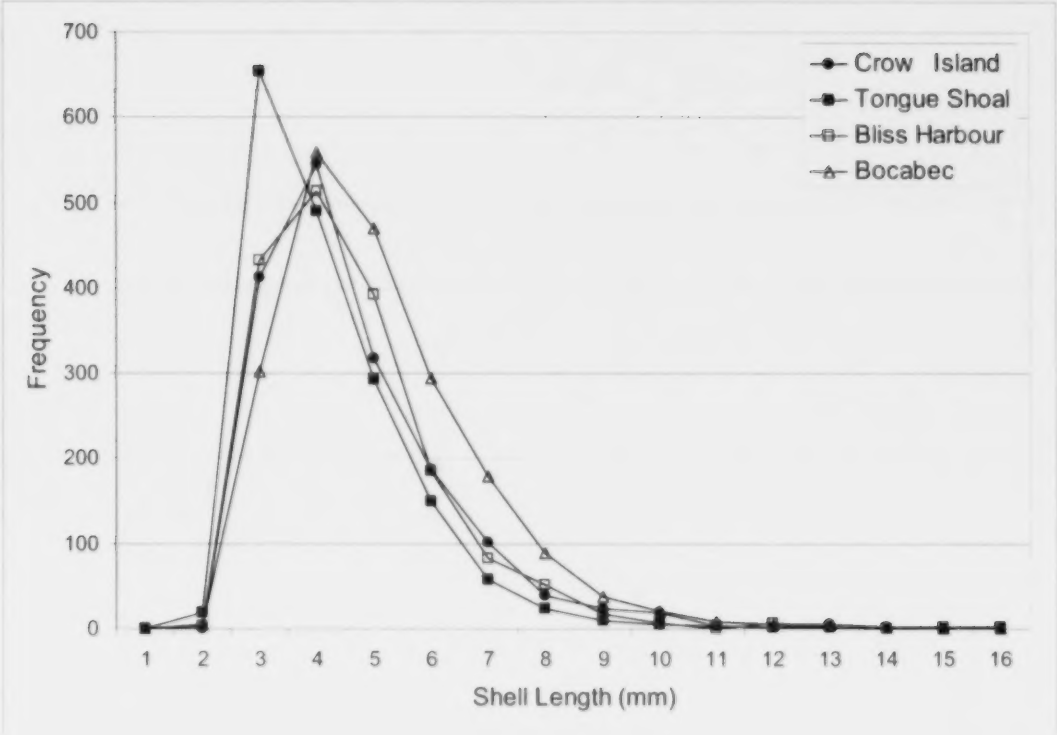

Figure 10: Shell length frequency distribution for 4 locations in the SW Bay of Fundy. Frequency values represent a sub sample of all treatments collected from each location in January 2007 after a period of approximately 5 months (n = 18 for each location).

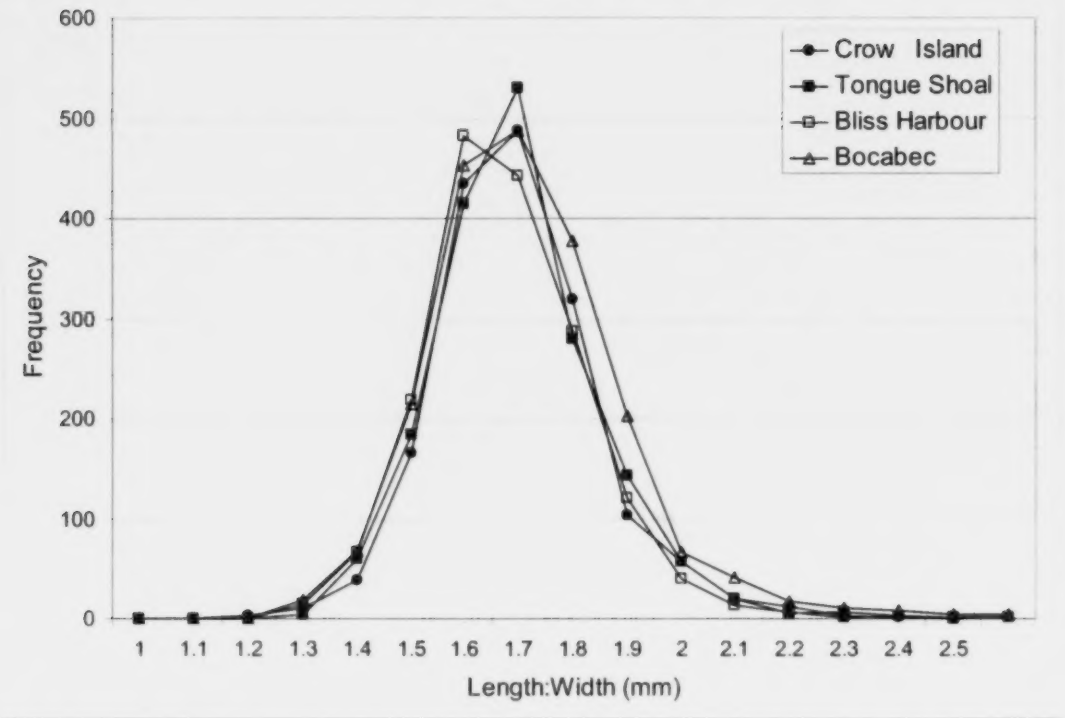

Figure 11: Length: width ratio frequency distribution for 4 locations in the SW Bay of Fundy. Frequency values represent a sub sample of all treatments collected from each location in January 2007 after a period of approximately 5 months (n=18 for each location).